

NEPHRIDIA IN THE LARVAE OF HEMICHORDATES AND ECHINODERMS

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ABSTRACT

The pore canal-hydropore complex in the larvae of echinoderms and hemichordates has long been recognized as an important character establishing a close phylogenetic relationship between the two phyla. An experimental and ultrastructural analysis of this complex in a tornaria and a bipinnaria larva indicates that it is a functional nephridium. The ciliated pore canal drives a constant, unidirectional efflux of coelomic fluid out of the hydropore. Two percent and 14% of the body volume are cleared per hour at the hydropore by a tornaria of *Schizocardium brasiliense* and a bipinnaria of *Asterias forbesi*, respectively. Fluid recovery by the coelom is from the blastocoel, the presumptive blood vascular space, across basal lamina and podocytes lining the coelomic cavity suggesting that the discharged fluid is formed by ciliary-driven ultrafiltration. Although invertebrate deuterostomes are believed to lack discrete excretory organs, an analysis of the metamorphosis of the larval nephridia suggests that adult echinoderms and hemichordates possess functional metanephridial systems.

INTRODUCTION

A signal achievement of classical morphology was the recognition of a close relationship between hemichordates and echinoderms based on structural similarities of their larvae (Gemmill, 1914; van der Horst, 1939). The most striking of these similarities is the pore canal-hydropore complex. It consists of a duct leading from a coelomic cavity to an external pore situated asymmetrically to the left of the dorsal midline as described in larvae of Enteropneusta and all five extant classes of echinoderms (Hyman, 1955, 1959). Yet despite the careful attention paid to this organ by morphologists of the nineteenth and early twentieth centuries, only one, T. H. Morgan, commented on its function. He speculated that "The whole structure is suggestive of an excretory arrangement [in the tornaria] . . ." (Morgan, 1894).

Invertebrate deuterostomes are generally believed to lack discrete nephridial organs and nowhere is the belief more frequently advanced than in the Echinodermata (Bin-yon, 1966; Barnes, 1980). We have been studying the generality of a model that predicts the occurrence of functional metanephridia in organisms where there is the potential for ultrafiltration of vascular fluid into a coelomic cavity, *viz.*, in coelomates with a blood vascular system (Ruppert and Smith, 1985, material in prep.). Our attention was drawn to larval echinoderms and hemichordates because of reports of a contractile vesicle beating rhythmically adjacent to the coelom and pore canal of definitive larvae in the Ophiuroidea (Gemmill, 1918), Echinoidea (Bury, 1896), Asteroidea (Gemmill, 1914), and Enteropneusta (Morgan, 1894). This organization suggested that blastocoelic fluid could be pressure filtered across the wall of the coelom and pore canal, modified by the lining cells, and released at the hydropore.

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Objectives of this investigation were to determine the pattern of fluid flow in the pore canal in the larvae of the asteroid, *Asterias forbesi*, and the enteropneust, *Schizocardium brasiliense*, and to identify potential sites of ultrafiltration using transmission electron microscopy (TEM). Because it soon became apparent that the larval complexes functioned as nephridia, another objective was to re-examine the adult derivatives of the larval structures to determine if adult enteropneusts and echinoderms might express functional nephridia.

MATERIAL AND METHODS

Collections of adult animals were made in March 1985 from a rock jetty at Murrell's Inlet, South Carolina (*Asterias*), and from a mudflat at North Inlet near Georgetown, South Carolina (*Schizocardium*) (Fox and Ruppert, 1985). Tornaria larvae of *Schizocardium* and bipinnaria larvae of *Asterias* were reared from eggs spawned in the laboratory. Cultures were maintained under constant agitation at 16°C for approximately 6 months with thrice weekly changes of natural seawater (33‰) and the addition of a few squirts from a Pasteur pipet of *Dunaliella salina* and *Isochrysis galbana*, as food.

Flow direction and flow rates in the ciliated pore canal were determined by microperfusion of the adjoining coelom with 0.45 μm latex microspheres (Polysciences), stabilized with BSA (Sigma), and suspended in seawater. These were introduced through the hydropore without damage to the larva. Particle movements were observed directly under a compound microscope (Zeiss Photomicroscope I) and recorded using cinematography. Cinemicrographic sequences were obtained on 16 mm film (Kodak Plus-X, Double-X, and Ektachrome 7240 color positive film) at 16 fps. Analyses of particle movement were made from prints of selected sequences and with the aid of a motion analyzer (Athena model NT-O).

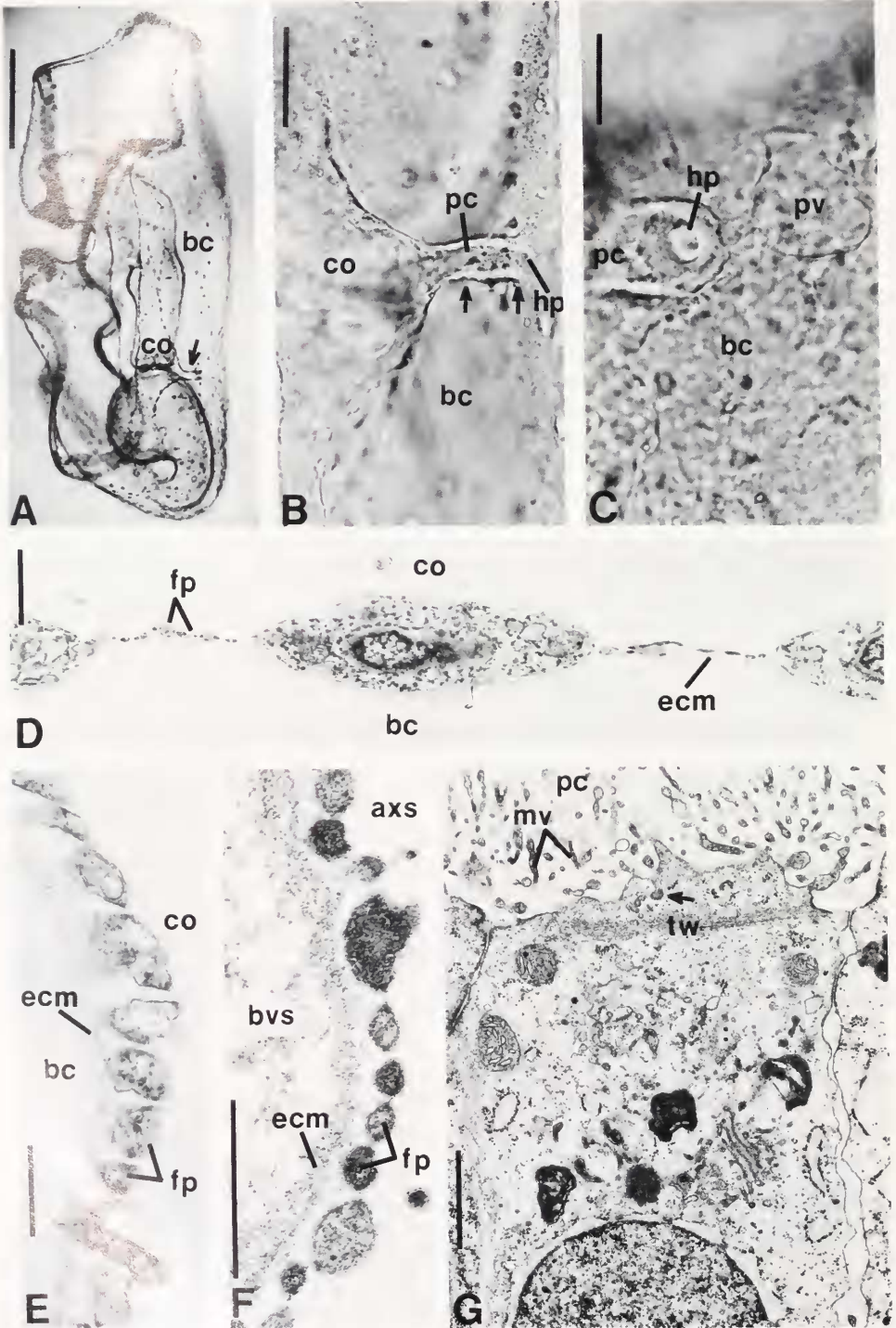
Volumes of internal cavities of both larvae were calculated from digitized tracings (HIPAD digitizer, Houston Instruments, Austin, Texas) of serial cross sections of specimens embedded in Polybed 812 (Polysciences). The computer analysis (Victor 9000, Victor Technologies, Scotts Valley, California) was based on an algorithm for the area of a polygon with vertices (Pearson, 1974).

Morphological data were obtained from living larvae and adults, plastic embedded specimens (Rieger and Ruppert, 1978), and thick sections drawn or photographed on a dissecting (Wild M-5) or compound microscope (Zeiss Photomicroscope I plus drawing tube). Specimens were fixed for thick sectioning and TEM (Philips EM 300) in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer for 1 h. Postfixation, after a brief buffer rinse, was in 1.0% OsO_4 in 0.2 M buffer for 1 h. After standard alcohol dehydration, the specimens were embedded in Polybed 812.

RESULTS

Development of the complex

Within 72 hours of fertilization at 16°C, the larvae of both species are swimming and possess a ciliated pore canal and dorsal hydropore joining a coelomic cavity to the exterior (Figs. 1A, 2A). A spherical vesicle develops beside the hydropore in the tornaria and near the right coelom in the bipinnaria about 3 weeks into larval life. The vesicle, a coelomic cavity, increases in size as it migrates through the blastocoelic jelly to graft, along two of its sides, near the junction of the pore canal and its coelom (Figs. 1C, 2C). An open cavity, continuous with the blastocoel, remains between one



wall of the vesicle and the pore canal coelom (Fig. 2C). Once in place, the vesicle undergoes a contraction every 5–10 seconds.

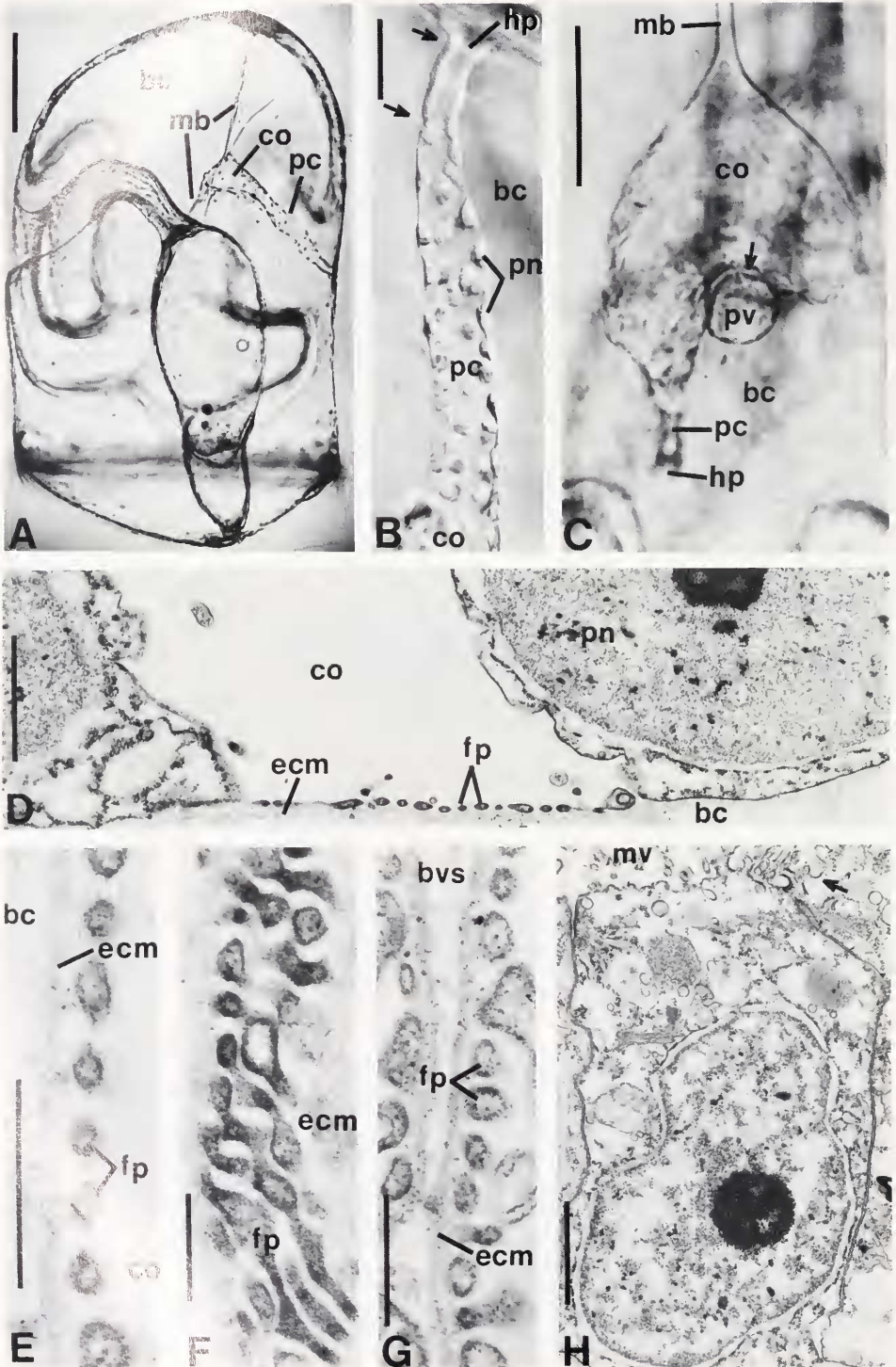
Fluid transport

Direct microscopic examination and cinemicrography revealed a continuous, ciliary-driven efflux of particles from the coelom and pore canal at the hydropore (Fig. 3). All cells forming the pore canal are ciliated as are most of the peritoneal cells lining the coelomic cavities. Table I contains morphometric data for the two larvae. The calculated average particle velocity was $46 \mu\text{m} \cdot \text{s}^{-1}$ (± 6.25 SE, $n = 4$, tornaria) and $88 \mu\text{m} \cdot \text{s}^{-1}$ (± 8.9 SE, $n = 2$, bipinnaria) in the ectodermal portion of the pore canal. From the dimensions of these cylindrical canals, calculations were made of the fluid volume cleared at the hydropore. Assuming a parabolic velocity profile across the pore canal, the volume cleared is $5.4 \text{ nl} \cdot \text{h}^{-1}$ by the tornaria and $27 \text{ nl} \cdot \text{h}^{-1}$ by the bipinnaria. At the calculated rate of discharge through the hydropore, the tornaria will replace its coelomic volume once every 13 min and the bipinnaria every 56 min. Because the coeloms do not collapse with the constant hydroporic efflux, fluid must be recovered from the blastocoel across the coelomic epithelium. Such a flux across the coelomic epithelium raises the possibility of a ciliary-driven ultrafiltration of blastocoelic fluid. Given blastocoelic volumes of $0.27 \mu\text{l}$ and $0.14 \mu\text{l}$ in the tornaria and bipinnaria, the entire blastocoelic volume could be cleared in 50 h and 5 h, respectively. The percent body water discharged per hour is approximately 2% for the tornaria and 14% for the bipinnaria.

Ultrastructure of the filtration surface

Transmission electron microscopy of the coelomic lining of the anterior coelom and mesodermal part of the pore canal in the early tornaria reveals that the entire lining is composed of podocytes lying on a thin basal lamina except for a few myoepithelial cells forming the apical muscle band (Fig. 2B, D, E, F). In the definitive tornaria, the podocytic lining is restricted to the region of contact between the coelom and pulsatile vesicle, and along the proximal portion of the pore canal (Fig. 2C). The remainder of the lining differentiates as myoepithelial cells that will contribute to the musculature of the adult proboscis. In the early and definitive bipinnaria, portions of the left coelom at the level of the hydropore are also lined by podocytes, especially along the medial and mediodorsal walls (Fig. 1D, E). Other coelomic regions have not been surveyed.

FIGURE 1. Hydropore-pore canal complex of the bipinnaria larva of *Asterias forbesi* (Asteroidea). (A) Left lateral view of living early larva showing left coelom (co), blastocoel (bc), and pore canal (arrow; bar = $200 \mu\text{m}$). (B) Lateral view of pore canal (pc) traversing blastocoel (bc) from left coelom (co) and opening dorsally at the hydropore (hp). Arrows define limit of ectodermal part of canal (bar = $50 \mu\text{m}$). (C) Dorsal view of pore canal (pc), hydropore (hp), and pulsatile vesicle (pv) in the blastocoel (bc). Complex is not yet fully organized. Top of micrograph is anterior (bar = $30 \mu\text{m}$). (D) TEM of peritoneal podocytes from medial wall of left coelom at the level of the pore canal. The basal lamina (ecm) is the only continuous barrier between the blastocoelic (bc) and coelomic (co) compartments (fp, foot processes of podocytes; bar = $1.0 \mu\text{m}$). (E) TEM of podocyte foot processes (fp) from same anatomical region as in D (bc, blastocoel; co, left coelom; ecm, basal lamina; bar = $0.5 \mu\text{m}$). (F) TEM of a portion of an axial gland blood vessel (bvs) of a juvenile *Asterias* (see Fig. 4; axs, axial sinus coelom; ecm, basal lamina; fp, foot processes of peritoneal podocytes; bar = $0.5 \mu\text{m}$). (G) TEM of columnar cell from ectodermal part of larval pore canal (pc). Each cell bears a single cilium (not shown) projecting into the pore canal (arrow = coated pit; mv, microvilli; tw, terminal web; bar = $1.0 \mu\text{m}$).



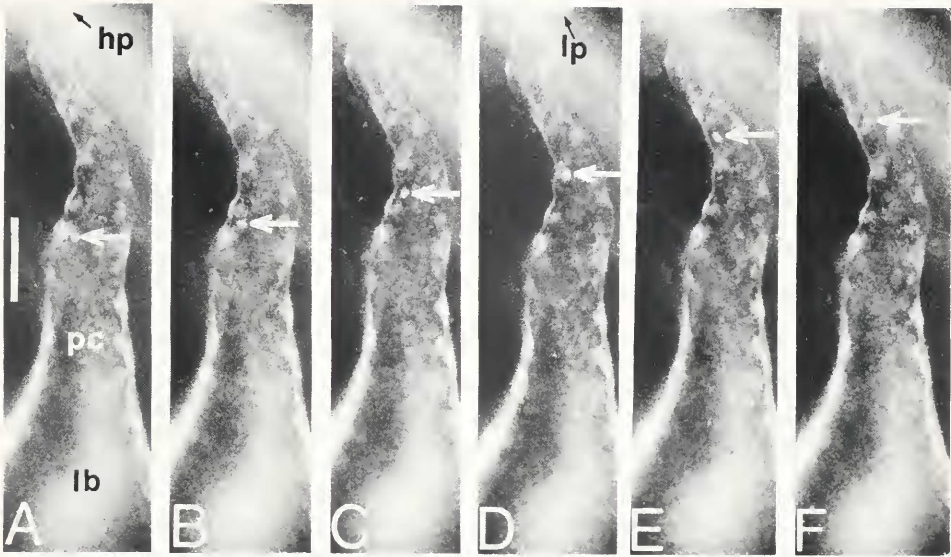


FIGURE 3. Ciliary transport of microbeads in the pore canal of *Schizocardium brasiliense* (Enteropneusta). (A-F) Cinemicrographs of a clump of microbeads in the mesodermal part of the pore canal (arrows; 0.25 s interval between frames). Velocity measurements for the calculations in the text were obtained only for the ectodermal part of the canal (hp, hydropore; lb, latex microbeads in anterior coelom; lp, plume of microbeads expelled at hydropore; pc, pore canal; bar = 50 μ m).

The ectodermally derived part of the pore canal adjoining the hydropore in both species is organized as a cuboidal to columnar, ciliated epithelium (Figs. 1G, 2H). The microvillar density and vesicular content of these cells are significantly higher than those associated with the adjacent, overlying, squamous larval epidermis.

The metamorphosis of the larval nephridia is described in Figure 4.

DISCUSSION

The results suggest that larval enteropneusts, asteroid, and perhaps larval echinoderms of the remaining four classes, all of which develop pore canal complexes (Hyman, 1955), possess a functional nephridium probably involved in extracellular volume regulation. The calculated values of 2% and 14% of the body volume cleared

FIGURE 2. Hydropore-pore canal complex of the tornaria larva of *Schizocardium brasiliense* (Enteropneusta). (A) Left lateral view of living early larva (bc, blastocoel; co, anterior coelom; mb, muscle bands; pc, pore canal; bar = 200 μ m). (B) Lateral view of pore canal (pc) traversing blastocoel (bc) and opening dorsally at the hydropore (hp). Arrows indicate ectodermal portion of canal (co, anterior coelom; pn, podocyte nuclei; bar = 40 μ m). (C) Dorsal view of definitive complex (anterior is toward top). The spherical pulsatile vesicle (pv) is joined laterally to the wall of the anterior coelom (co) enclosing a small cavity (arrow) that is continuous with the blastocoel (bc; hp, hydropore; mb, muscle band; pc, pore canal; bar = 100 μ m). (D) TEM of peritoneal podocytes (pn) from proximal pore canal. The podocyte basal lamina (ecm) is the only continuous barrier between the blastocoelic (bc) and coelomic (co) compartments (fp, foot processes; bar = 1.0 μ m). (E-F) Transverse and grazing TEM sections of larval podocytes (bars = 0.5 μ m). TEM of podocyte foot processes (fp) on small blood vessels (bvs) in the glomerulus of the enteropneust *Saccoglossus kowalevskii* (see Fig. 4; ecm, basal lamina; bar = 0.5 μ m). (H) TEM of columnar cell from ectodermal part of larval pore canal. Each cell bears more than one cilium (not shown) projecting into the pore canal (arrow = coated pit; mv, microvilli; bar = 1.0 μ m).

TABLE I

Morphometric data of the larvae of Schizocardium brasiliense and Asterias forbesi (volumes in μl)

Larva	Tornaria	Bipinnaria
Total length (mm)	1.1	1.6
Body vol.	2.9×10^{-1}	1.9×10^{-1}
Blastocoel vol.	2.7×10^{-1}	1.3×10^{-1}
Protoceol vol.	1.2×10^{-3}	—
Pulsatile vesicle vol.	1.1×10^{-4}	1.3×10^{-4}
Enterocoel vol.	—	2.6×10^{-2}
Left mesocoel vol.	2.8×10^{-4}	—
Right mesocoel vol.	4.0×10^{-4}	—
Left metacoel vol.	6.6×10^{-4}	—
Right metacoel vol.	7.2×10^{-4}	—
Gut vol.	1.5×10^{-2}	3.0×10^{-2}
Ectodermal pore canal length (μm)	45	35
Ectoderm pore canal diameter (μm)	9	15

per hour by these larvae fall within or close to the general range of 1% to 10% given by Kirschner (1967) for aquatic animals. It should be noted, however, that the values presented here are rough estimates based on measurements of the few particles that remained in the focal plane of the microscope while in transit along the pore canal. Given the constant hydroporic efflux, fluid recovery by the adjoining coelom must be from the surrounding blastocoel across the layer of continuous basal lamina and peritoneal podocytes. The mechanism of fluid recovery across the body wall has not been addressed in this study. We speculate that it is driven by an osmotic gradient (Oglesby, 1981), perhaps established by proteins in the blastocoelic jelly.

A protonephridium can be defined functionally as an excretory organ where filtration occurs on the nephridium (terminal cell) and is driven by cilia whereas, in metanephridial systems, filtration occurs on blood vessels or their analogues and filtration pressure is muscular in origin (Ruppert and Smith, 1985, material in prep.). If invertebrate nephridia are so defined, then the pore canal-hydropore complex of these larvae can be considered as a functional protonephridium with the ciliated cells of the pore canal forming the pump driving filtration. It is possible that, when the pulsatile vesicle is positioned, the system functions as a metanephridium, with the ciliary pump being augmented by a muscular pump, the pulsatile vesicle. Superseding of a larval protonephridium by a later metanephridium is a common feature of representatives in several phyla, *e.g.*, Annelida (Goodrich, 1945), Phoronida (Emig, 1982), and Mollusca (Brandenburg, 1966).

By following the transformation of the larval nephridium during metamorphosis (summary Fig. 4), it is possible to predict that adult asteroids and enteropneusts also possess discrete, functional nephridia. The heart complex, situated in the proboscis of adult enteropneusts, has the structural components of a metanephridial system, including a capillary bed overlain with podocytes associated with the heart ("glomerulus"; Fig. 2G; Wilke, 1971). Modification of the ultrafiltrate could occur in the proboscis coelom, thus delivering nutrients to the proboscis musculature, a tissue with a poorly developed vascular supply. Some modification may also occur along the proboscis duct before a fluid is discharged by the ciliary excurrent at the proboscis pore (Balser, 1985, material in prep.).

The axial complex in adult asteroids, associated with the madreporite, also has the structure of a metanephridial system. We speculate that vascular fluid in the cap-

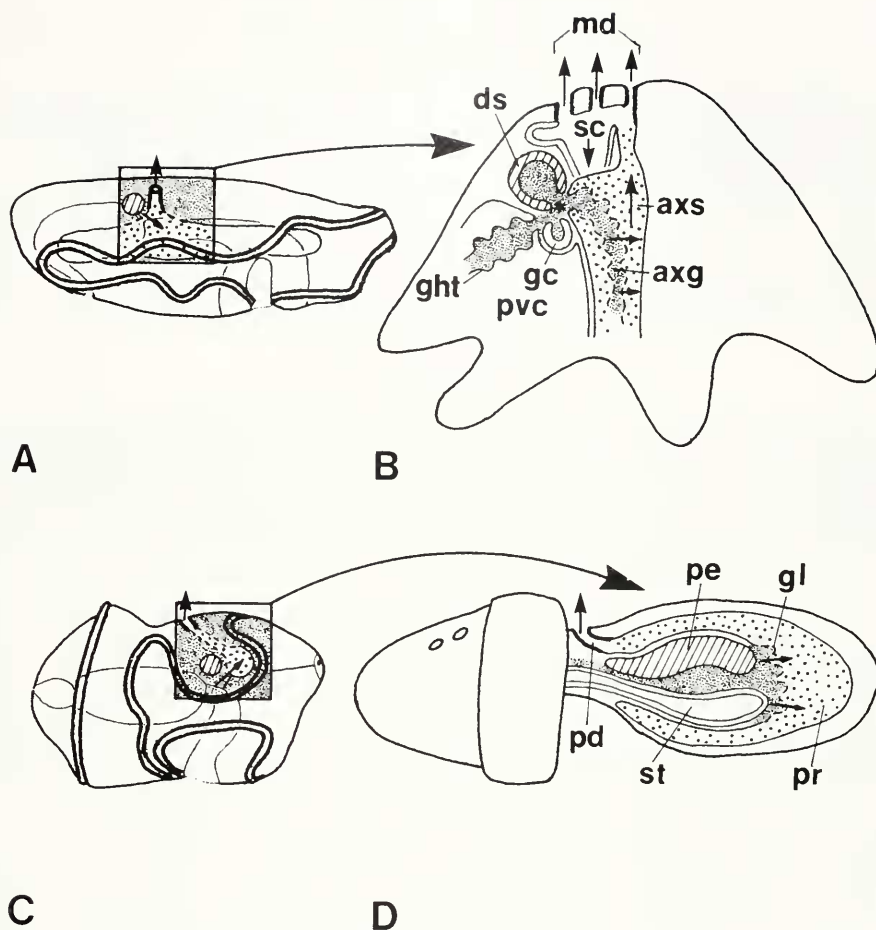


FIGURE 4. Comparison of larval nephridia with adult derivatives. The following conventions apply throughout: small arrows = presumed direction of filtration, larger arrows = observed direction of ciliary beat, cross hatching = pulsatile vesicles in larvae and their derivatives in the adults [madreporic vesicle or dorsal sac (*ds*) in *Asterias*, pericardium (*pe*) in *Schizocardium*], dashed lines = coelomic epithelia composed of podocytes, loose stipple = anterior larval coeloms and their adult derivatives (axial sinus (*axes*) of *Asterias* and proboscis coelom (*pr*) of *Schizocardium*), fine stipple = blastocoel of larvae and their adult derivative, the blood vascular system, bold lines = larval ectodermal pore canals and adult derivatives [pore canals of the madreporite (*md*) of *Asterias* and the proboscis duct (*pd*) of *Schizocardium*]. (A) Lateral view of the bipinnaria of *Asterias* showing nephridial organs. (B) Disproportionately enlarged apical portion of axial complex of adult *Asterias* illustrating anatomical relationships of major components as viewed in vertical section: madreporite (*md*), madreporic vesicle (*ds*), stone canal (*sc*), axial sinus (*axes*), axial gland (*axg*), gastric haemal tuft (*ght*), genital coelom (*gc*), and perivisceral coelom (*pvc*). The madreporic vesicle is contractile like its ontogenetic precursor, the pulsatile vesicle. Both the gastric haemal tuft (*ght*) and axial gland (*axg*) are tangles of small blood vessels. The gastric haemal tuft spans the perivisceral coelom (*pvc*) to join the major vessels of the gut while the axial gland vessels join the hyponeural sinus vessels, a portion of the blood vascular system (BVS) and its surrounding coelom that parallels the distribution and extent of the nervous system. The genital coelom (*gc*) and the BVS it encloses form a ring at the aboral surface of the disc. Vessels from the gut, genital, and peripheral parts of the BVS communicate directly (asterisk) with the vascular cavity ("head process") of axial gland enclosed by the contractile madreporic vesicle (*ds*). Pressure for filtration of vascular fluid in the axial gland vessels across the ECM and podocytes of the axial sinus coelom (*axes*) may be generated by the madreporic vesicle or perhaps by contractions of vessels within the axial gland itself. (C) Lateral view of the tornaria of *Schizocardium* showing nephridial organs. (D) Disproportionately enlarged proboscis of adult *Schizocardium* illustrating anatomical relationships of major components: proboscis duct (*pd*), pericardium (*pe*), glomerulus (*gl*), proboscis coelom (*pr*), and stomochord (*st*). The pericardium (*pe*) is contractile like its ontogenetic precursor, the pulsatile vesicle. Pressure for filtration of vascular fluid from the heart and glomerular vessels across the ECM and podocytes of the proboscis coelom may be generated by the pericardium or by contraction of vessels entering the central sinus of the heart.

illary bed of the axial gland is pressure filtered across the layer of peritoneal podocytes (Fig. 1F; Bargmann and von Hehn, 1968) into the axial sinus (a coelomic cavity) where modification could occur. A fluid could be discharged by the ciliary excurrent from the pores of the madreporite.

The recognition of discrete excretory organs in larval hemichordates and echinoderms was prompted by a consideration of a general model for nephridial design and function (Ruppert and Smith, 1985, in prep.). We anticipate that further experimental investigations of other predictions in the model will lead to similar discoveries and an enhanced understanding of renal function and nutrient translocation in animals.

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